



# Evaluation of The Anticancer Activity of Hydroxyxanthenes Against Human Liver Carcinoma Cell Line

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## Abstract

Nowadays, cancer is one of the most fatal diseases in developed and developing countries. Therefore, it is an urgent need to find more effective anticancer drugs among the recent commercially available standard drugs. Xanthone derivatives have been researched as anticancer drugs due to their ease of synthesis and structure modification, as well as their excellent anticancer activity. In this work, the *in vitro* anticancer activity of hydroxyxanthenes against the human liver carcinoma cell line (HepG2) was evaluated. Among the twenty-two hydroxyxanthenes, 1,3,6,8-tetrahydroxyxanthone was found as the most active anticancer agent with an  $IC_{50}$  value of 9.18  $\mu$ M, which was better than doxorubicin as the standard drug. From the molecular docking studies against topoisomeraseII $\alpha$  and two c-KIT protein kinases, 1,3,6,8-tetrahydroxyxanthone yielded strong binding energy in a range of -25.48 to -30.42 kJ/mol. The 1,3,6,8-tetrahydroxyxanthone could bind on the active site of these protein receptors through hydrogen bonds with key amino acid residues (Glu640, Cys673, Gln767, Met769, Asp810, and Asp831), as well as nitrogen bases (Adenine12 and Guanine13), thus leading to the death of HepG2 cancer cells through the apoptosis mechanism.

**Keywords:** anticancer; human liver carcinoma cell line; hydroxyxanthone; molecular docking

## 1. INTRODUCTION

According to the World Health Organization report, cancer is awarded as the deadliest disease. It was estimated that one in six deaths in the world is caused by cancer disease. In 2008, around 12.6 million people were infected by cancer. This number kept the increase to 18.1 million in 2018 and is estimated to reach 29.4 million in 2040 [1]. Among the cancer diseases, liver cancer ranked among the top three causes of cancer death in 46 countries in 2020 due to its very high mortality rate. Rumgay et al. [2] reported that 905,700 people were diagnosed with liver cancer in 2020 and 830,200 people died from liver cancer in the same year. It meant the mortality rate of liver cancer reached 91.66%, which was a very serious issue. Additionally, they estimated that the number of liver cancer death cases could increase to more than 1,286,810 if the recent death rate is not changed. Therefore, there is no reason to not giving a serious

effort to decrease the number of liver cancer active cases and its mortality rate in the future.

A number of standard anticancer drugs to cure and treat liver cancer have been commercially available nowadays. Among them, doxorubicin is one of the most used anticancer drugs [3]. However, doxorubicin resistance has been reported in this century, and doxorubicin has failed to give any clinical efficacy as a systemic treatment for human liver cancer cells [4]. Doxorubicin has an anthracycline structure that is able to interact with c-KIT protein kinase (epidermal growth factor receptor (EGFR) and platelet-derived growth factor (PDGFR)) and topoisomeraseII $\alpha$  (TopII $\alpha$ ) protein receptors. TopII $\alpha$  catalyzes DNA replication and transcription of cancer cells [5]. When the doxorubicin interacts with the DNA strain of the TopII $\alpha$  protein, the protein synthesis process in the cancer cells will be interrupted, thus, activating the p53 nuclear transcription factor and changing the ratio of pro- and anti-apoptotic Bcl-2 proteins. These phenomena lead to the apoptosis and death of cancer cells [6]. EGFR protein receptor plays an important role in cancer cell signaling pathways that control cancer cell survival, differentiation, and proliferation [7]-[9], while PDGFR protein regulates the cancer cell migration, survival, and proliferation [10]-[12]. When these protein receptors are inhibited, the cancer cells can not be spread out and multiplied, thus leading to the death of cancer cells. This mechanism is a useful insight for the design and development of new liver

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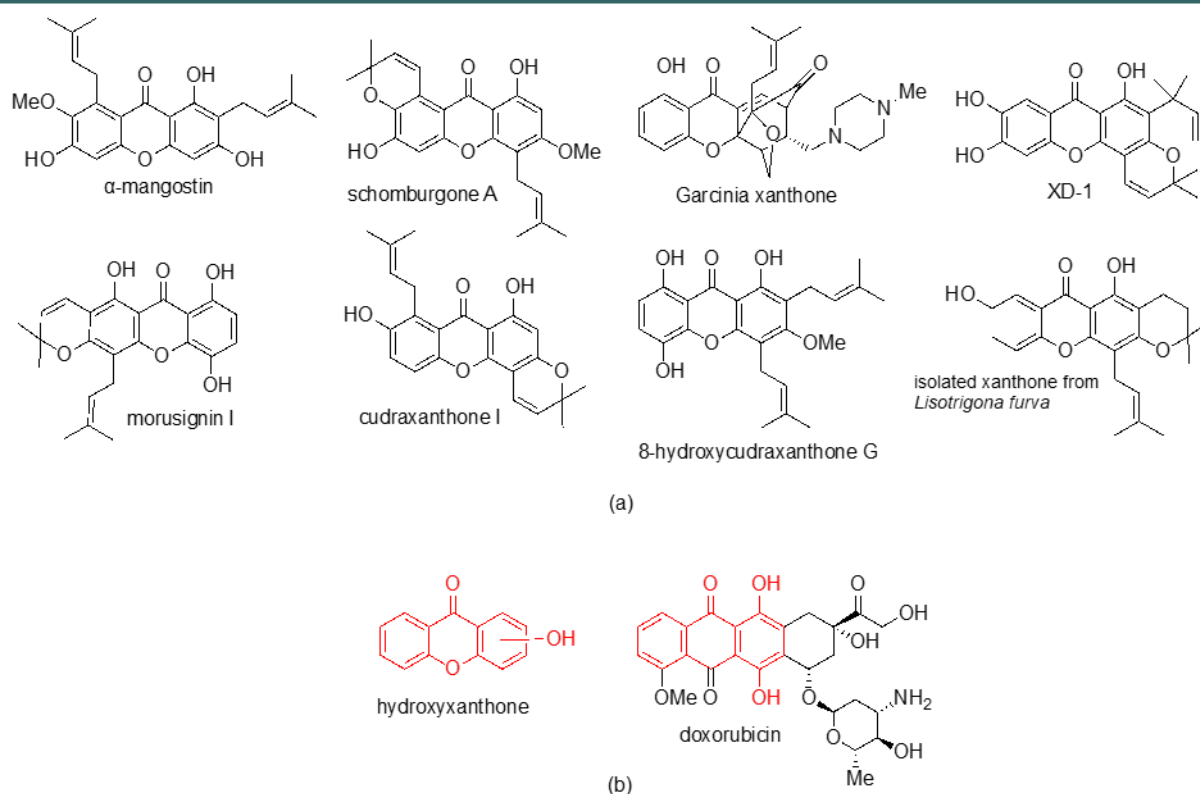
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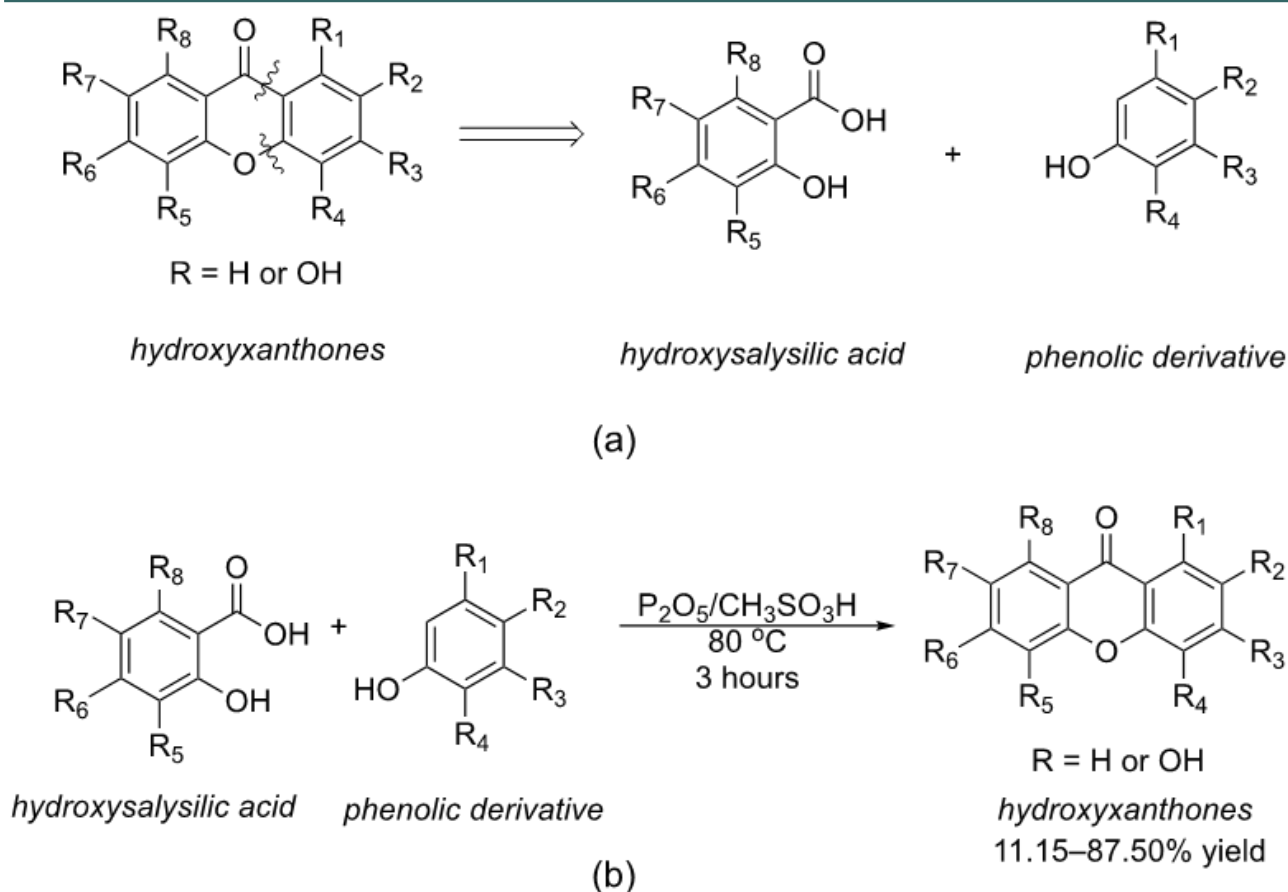


**Figure 1.** (a) The chemical structures of natural xanthenes. (b) The structural similarity between doxorubicin and hydroxyxanthone

anticancer drugs to replace the use of doxorubicin in the future.

Hundreds of anticancer drugs have been designed and developed over the past several years [13][14]. Among them, xanthone derivatives show potential anticancer activity through *in vitro*, *in vivo*, and even clinical trials [15]. With a simple chemical structure, the xanthone derivative is able to bind with several protein receptors, thus exhibiting a wide spectrum of anticancer agents depending on the position, number, and type of attached functional groups. Natural xanthenes, such as  $\alpha$ -mangostin, schomburgone A, Garcinia xanthone, XD-1, morusignin I, cudraxanthone I, 8-hydroxycudraxanthone G, and xanthone from *Lisotrigona furva*, have been isolated and examined against human liver carcinoma cell line (HepG2) with *in vitro* half-maximal inhibitory concentration ( $IC_{50}$ ) value of 242.58, 45.05, 3.25, 18.60, 70.38, 9.63, 39.22, and 33.20  $\mu$ M, respectively [16]–[19]. Their chemical structures are shown in Figure 1(a). However, the isolation of natural xanthenes is laborious work as the isolation yield sometimes does not exceed 0.1% [20].

Hydroxyxanthone, a family of simple-oxygenated xanthone, is the most investigated xanthone derivative as an anticancer agent due to its ease of synthesis, simple purification, moderate to high synthetic yield, and active to several cancer cell lines [15]. The presence of the hydroxyl group is also confirmed in the reported natural xanthenes (Figure 1(a)). Furthermore, the structure of hydroxyxanthone has a similarity to the doxorubicin thus, the hydroxyxanthone may work in a similar mechanism to the doxorubicin (Figure 1(b)). Unfortunately, to the best of our knowledge, an evaluation of the number and position of hydroxyl groups of hydroxyxanthenes with their anticancer activity against the HepG2 cancer cell line is rarely reported. Therefore, in this work, we summarized the anticancer activity of hydroxyxanthenes from our previous work and other reported literatures and discussed the effect of the number and position of hydroxyl groups with their anticancer activity against HepG2 cancer cell line. Additionally, we conducted an *in silico* approach through molecular docking studies of the most active hydroxyxanthone against TopII $\alpha$  and two c-KIT protein kinases,



**Figure 2.** (a) The retrosynthetic analysis and (b) the general synthesis of hydroxyxanthones

named EGFR and PDGFR receptors, to elucidate its mechanism of action as the anticancer agent against HepG2 cancer cell line.

## 2. MATERIALS AND METHODS

### 2.1. Materials

The chemical structure and anticancer activity of xanthone, 1-hydroxyxanthone, 3-hydroxyxanthone, 1,3-dihydroxyxanthone, 1,6-dihydroxyxanthone, 3,6-dihydroxyxanthone, 1,3,6-trihydroxyxanthone, 1,3,7-trihydroxyxanthone, 1,3,8-trihydroxyxanthone, and 1,3,6,8-tetrahydroxyxanthone have been reported in our previous work [15][21]–[27]. Meanwhile, the chemical structure and anticancer activity of the other hydroxyxanthones were obtained from the reported publications [28]–[31].

The three-dimensional crystallography structure of TopII $\alpha$ , EGFR, and PDGFR receptors together with their native ligands, i.e., mitoxantrone,

erlotinib, and imatinib, was downloaded from Protein Data Bank ([www.rcsb.org](http://www.rcsb.org)) with PDB ID of 1M17, 1T46, and 4G0V, respectively. The used software for molecular docking studies, i.e., Chimera 1.13.1, Gaussian09W, AutoDockTools-1.5.6, and Discovery Studio Visualizer 2019, were available in Austrian-Indonesia Center for Computational Chemistry, Department of Chemistry, Universitas Gadjah Mada, Indonesia.

### 2.2. Methods

#### 2.2.1. Molecular docking of hydroxyxanthones as anticancer agents

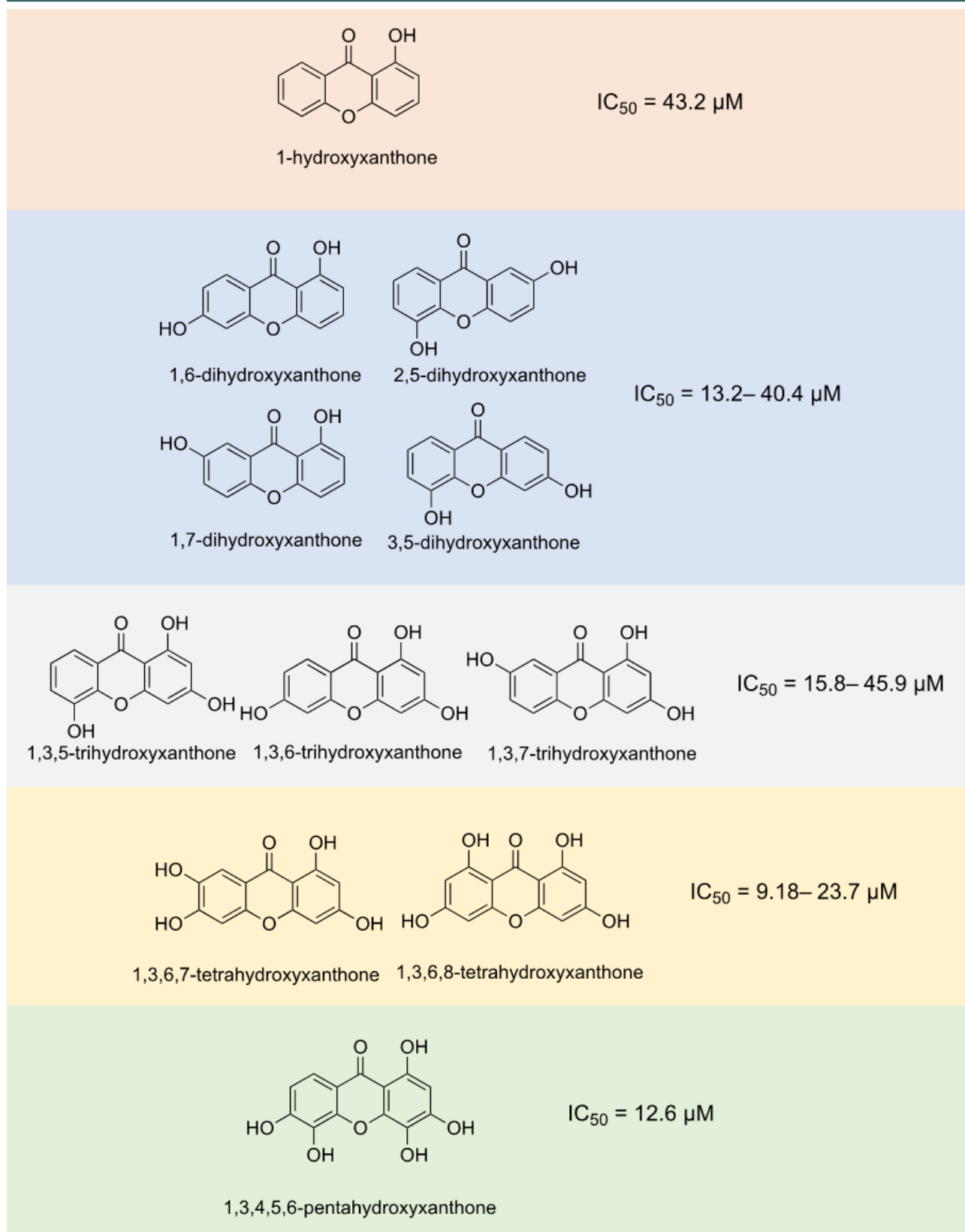
The molecular docking of hydroxyxanthones as an anticancer agent was performed through four steps, i.e., preparation of protein receptor and native ligand, geometry optimization of hydroxyxanthone, re-docking of native ligand, and docking of hydroxyxanthone derivative. First, each protein receptor was separated from its native ligand using Chimera 1.13.1 software. The water molecules were

also removed, and then each protein receptor and native ligand was saved in pdb format. Second, the three-dimensional structure of hydroxyxanthone was built using Gaussian09W software. Then, the structure of hydroxyxanthone was optimized using a Density Functional Theory-B3LYP method with a basis set of 6,31G. The optimized structure was also saved in pdb format. Third, the re-docking process is conducted using AutoDockTools-1.5.6 software in a grid box with a dimension of 50×50×50 Å and spacing of 0.375 Å for 100 runs of Lamarckian

Genetic Algorithm. The native ligand and protein receptor were fixed as flexible and rigid forms, respectively, during the re-docking process. The used parameters were valid when the root-mean-square deviation (RMSD) was less than 2.00 Å [32]. When this condition was achieved, the re-docking parameters were saved and used for the docking of hydroxyxanthone. Finally, the hydroxyxanthone was docked on the same position of the native ligand for each protein receptor with exactly the same parameters as the re-docking

**Table 1.** Anticancer activity of hydroxyxanthenes against HepG2 cancer cell line

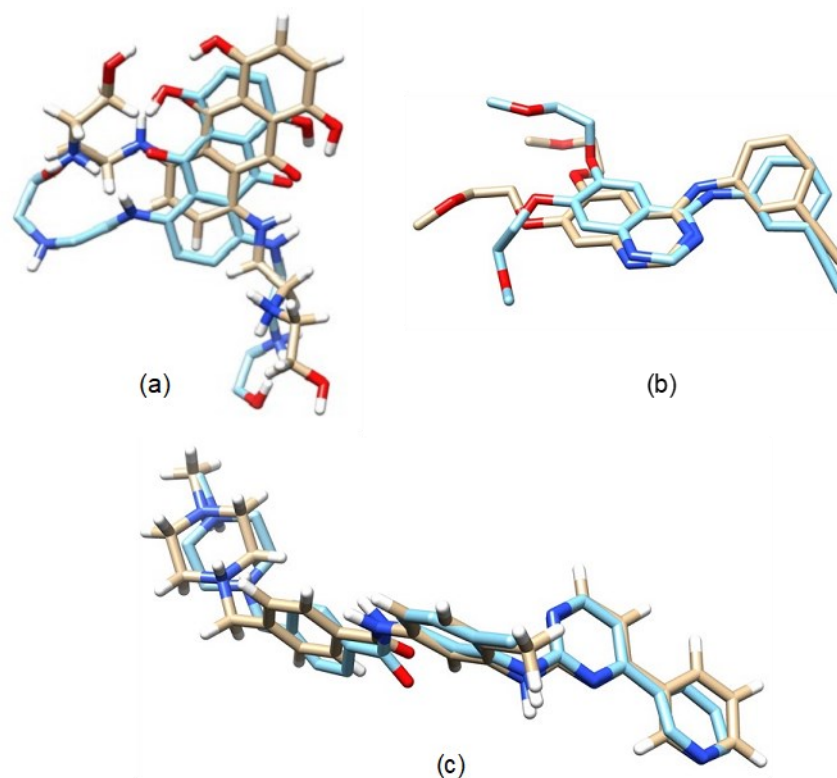
No	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>	R <sub>5</sub>	R <sub>6</sub>	R <sub>7</sub>	R <sub>8</sub>	IC <sub>50</sub> (μM)
1	H	H	H	H	H	H	H	H	85.3
2	OH	H	H	H	H	H	H	H	43.2
3	H	H	OH	H	H	H	H	H	85.3
4	OH	H	OH	H	H	H	H	H	71.4
5	OH	H	H	H	H	OH	H	H	40.4
6	OH	H	H	H	H	H	OH	H	13.2
7	H	OH	H	H	OH	H	H	H	23.8
8	H	OH	H	H	H	OH	H	H	52.2
9	H	OH	H	H	H	H	OH	H	>200
10	H	H	OH	OH	H	H	H	H	89.7
11	H	H	OH	H	OH	H	H	H	23.7
12	H	H	OH	H	H	OH	H	H	61.7
13	OH	H	OH	H	OH	H	H	H	15.8
14	OH	H	OH	H	H	OH	H	H	45.9
15	OH	H	OH	H	H	H	OH	H	33.8
16	OH	H	OH	H	H	H	H	OH	63.1
17	H	OH	OH	H	H	H	OH	H	63.3
18	H	H	OH	OH	H	OH	H	H	87.3
19	H	H	OH	OH	H	H	OH	H	>200
20	OH	H	OH	H	H	OH	OH	H	23.7
21	OH	H	OH	H	H	OH	H	OH	9.18
22	OH	H	OH	OH	OH	OH	H	H	12.6
23	Doxorubicin								46.9



**Figure 3.** The chemical structure of potential hydroxyxanthones as the anticancer agent against HepG2 cancer cell line

process. The results of molecular docking studies, i.e., binding energy, binding constant, and RMSD

values of hydroxyxanthone derivative for each protein receptor. The formed interactions between



**Figure 4.** Superimposed three-dimensional structure of native ligand: (a) mitoxantrone, (b) erlotinib, and (c) imatinib. Light-brown color represents the original position of the native ligand, while the light-blue color represents the position of the native ligand after the re-docking process

hydroxyxanthone derivative with amino acid and/or nitrogen base residue(s) on each active site of the protein receptor were visualized using Discovery Studio Visualizer 2019 software.

### 3. RESULTS AND DISCUSSIONS

#### 3.1. Summary of the anticancer activity of hydroxyxanthones

Hydroxyxanthone is a subfamily of xanthone having a hydroxyl group(s) on its structure. It was reported that the hydroxyl group is critical for anticancer activity due to its ability to form hydrogen bonds with the active site of protein receptors inside the cancer cells [33]. In general, hydroxyxanthone could be obtained by a one-pot reaction between hydroxysalicylic acid and phenolic derivative, as suggested by the disconnection analysis on the C-C acylation and dehydration of ring-closure (Figure 2(a)). In the previous works, twenty-two hydroxyxanthones have been synthesized and obtained in 11.15–87.50% yield [21]–[31]. The *in vitro* MTT (3-(4,5-

dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was used to measure the HepG2 cancer cells' viability and the data were calculated using probit analysis to obtain the  $IC_{50}$  value. A higher  $IC_{50}$  value means it requires a higher concentration of drug compound to cause the death of 50% of the cancer cells' population. On the other way, a higher  $IC_{50}$  value means weaker anticancer activity [34]. The general structure and anticancer activity of hydroxyxanthones are shown in Table 1.

From Table 1, hydroxyxanthones gave anticancer activity against the HepG2 cancer cell line depending on the number and position of the hydroxyl group. Xanthone with no hydroxyl substituent gave the  $IC_{50}$  value of 85.3  $\mu$ M (Table 1 list no. 1) and was further used as the control to discuss the effect of the hydroxyl group. The addition of a hydroxyl group on the xanthone structure on the 3-position did not influence its anticancer activity ( $IC_{50}$  = 85.3  $\mu$ M, Table 1 list no. 3). However, a hydroxyl group on the 1-position increased the anticancer activity of xanthone to have an  $IC_{50}$  value of 43.2  $\mu$ M (Table 1 list no. 2). It



means that the hydroxyl group on 1-position is important on the anticancer activity of xanthone.

Further addition of a hydroxyl group on the 1-hydroxyxanthone yield 1,X-dihydroxyxanthone compounds (Table 1 list no. 4–6). Overall, the 1,X-dihydroxyxanthones, i.e., 1,3-dihydroxyxanthone ( $IC_{50} = 71.4 \mu M$ ), 1,6-dihydroxyxanthone ( $IC_{50} = 40.4 \mu M$ ) and 1,7-dihydroxyxanthone ( $IC_{50} = 13.2 \mu M$ ) gave stronger anticancer activity than xanthone with no hydroxyl substituent ( $IC_{50} = 85.3 \mu M$ ). Compared to the anticancer activity of 1-hydroxyxanthone ( $IC_{50} = 43.2 \mu M$ ), the 1,X-dihydroxyxanthones ( $IC_{50} = 13.2–71.4 \mu M$ ) gave stronger anticancer activity except for 1,3-dihydroxyxanthone.

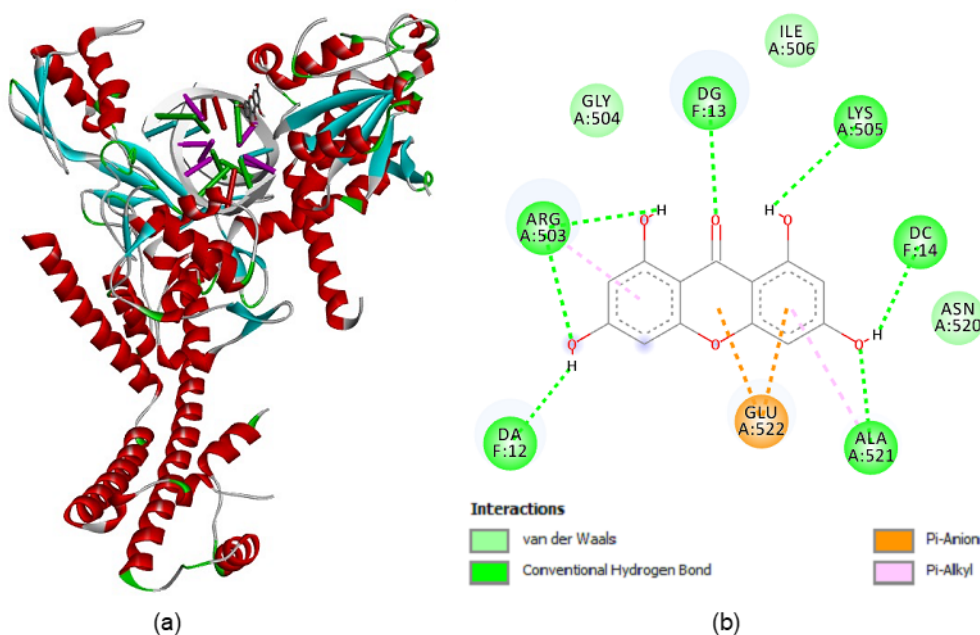
On the other hand, the 2,X-dihydroxyxanthones also gave stronger anticancer activity ( $IC_{50} = 23.8–52.2 \mu M$ , Table 1 list no. 7–9) than xanthone with no hydroxyl substituent except for 2,7-dihydroxyxanthone ( $IC_{50} > 200 \mu M$ ) indicating that 7-position is unfavorable for anticancer activity against HepG2 cancer cell line. Meanwhile, the 3,X-dihydroxyxanthone also gave higher anticancer activity ( $IC_{50} = 23.7–61.7 \mu M$ , Table 1 list no. 10–12) than xanthone with no hydroxyl substituent ( $IC_{50} = 85.3 \mu M$ ) and 3-hydroxyxanthone ( $IC_{50} = 85.3 \mu M$ ) except for 3,4-dihydroxyxanthone ( $IC_{50} = 89.7 \mu M$ ) indicating that additional hydroxyl group

at the 4-position was inactive as an anticancer drug.

Trihydroxyxanthones, xanthone derivatives with three hydroxyl groups, also gave stronger anticancer activity ( $IC_{50} = 15.8–63.3 \mu M$ , Table 1 list no. 13–19) against HepG2 cancer cell line compared with xanthone with no hydroxyl group except for 3,4,6-trihydroxyxanthone ( $IC_{50} = 87.3 \mu M$ ) and 3,4,7-trihydroxyxanthone ( $IC_{50} > 200 \mu M$ ). This result confirmed the other data that the hydroxyl group at the 4- and 7-position was not recommended for the liver cancer drug design based on the structure of xanthone derivatives.

The 2,3,7-trihydroxyxanthone gave stronger anticancer activity ( $IC_{50} = 63.3 \mu M$ ) than 2,7-dihydroxyxanthone ( $IC_{50} > 200 \mu M$ ) indicating that the hydroxyl group at 3-position is crucial for polyhydroxylated xanthone. Meanwhile, compared to 1,3-dihydroxyxanthone ( $IC_{50} = 71.4 \mu M$ ), the 1,3,5-trihydroxyxanthone, 1,3,6-trihydroxyxanthone, 1,3,7-trihydroxyxanthone, and 1,3,8-trihydroxyxanthone yielded higher anticancer activity with the  $IC_{50}$  value of 15.8, 45.9, 33.8 and 63.1  $\mu M$ , respectively. These results indicated that an additional hydroxyl group at the left aromatic ring of 1,3-dihydroxyxanthone structure enhanced its anticancer activity.

To expand our knowledge on the anticancer activity assay of hydroxyxanthones, further



**Figure 5.** (a) Three-dimensional and (b) two-dimensional structure of 1,3,6,8 tetrahydroxyxanthone on the active site of topoisomeraseII $\alpha$  protein receptor

**Table 2.** Molecular docking results of hydroxyxanthones against topoisomeraseII $\alpha$  and c-KIT protein kinase

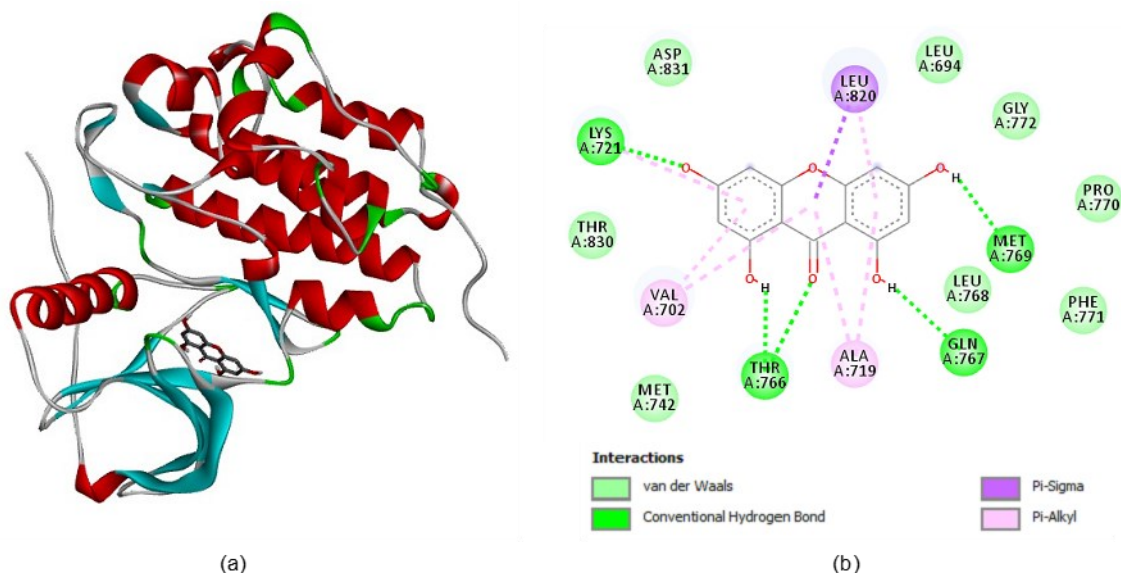
Protein Receptor	Binding energy (kJ/mol)	Binding constant ( $\mu$ M)	RMSD ( $\text{\AA}$ )	Hydrogen bond	van der Waals	Other interactions
TopII $\alpha$	-25.48	34.3	1.85	Adenine12, Guanine13, Cytosine14, Arg503, Lys505,	Gly504, Ile506, Asn520	Pi-anion: Glu522 Pi-alkyl: Arg503, Ala521
EGFR	-28.74	9.24	0.10	Lys721, Thr766, Gln767, Met769	Leu694, Met742, Leu768, Pro770, Phe771, Gly772, Thr830,	Pi-sigma: Leu820 Pi-alkyl: Val702, Ala719
PDGFR	-30.42	4.71	1.85	Thr670, Glu671, Cys673, Asp810	Lys623, Glu640, Gly676	Carbon hydrogen bond: Tyr672, Phe811 Pi-sigma: Leu595, Leu799 Pi-sulfur: Cys809 Pi-pi stacked and Pi-pi T-shaped: Tyr672, Phe811 Pi-alkyl: Val603, Ala621, Val654

hydroxylated of trihydroxyxanthone, i.e., tetrahydroxyxanthone and pentahydroxyxanthone was also evaluated (Table 1 list 20–22). Either 1,3,6,7-tetrahydroxyxanthone ( $IC_{50} = 23.7 \mu\text{M}$ ) or 1,3,6,8-tetrahydroxyxanthone ( $IC_{50} = 9.18 \mu\text{M}$ ) or 1,3,4,5,6-pentahydroxyxanthone ( $IC_{50} = 12.6 \mu\text{M}$ ) exhibit stronger anticancer activity than xanthone with no hydroxyl group ( $IC_{50} = 85.3 \mu\text{M}$ ), 1-hydroxyxanthone ( $IC_{50} = 43.2 \mu\text{M}$ ), 3-hydroxyxanthone ( $IC_{50} = 85.3 \mu\text{M}$ ), 1,3-dihydroxyxanthone ( $IC_{50} = 71.4 \mu\text{M}$ ), and 1,3,6-trihydroxyxanthone ( $IC_{50} = 45.9 \mu\text{M}$ ). The 1,3,6,7-tetrahydroxyxanthone ( $IC_{50} = 23.7 \mu\text{M}$ ) gave weaker anticancer activity against HepG2 cancer cell line than 1,3,6,8-tetrahydroxyxanthone ( $IC_{50} = 9.18 \mu\text{M}$ ) due to the presence of 7-hydroxyl which was inactive as aforementioned above. Meanwhile, the 1,3,4,5,6-pentahydroxyxanthone ( $IC_{50} = 12.6$

$\mu\text{M}$ ) yielded a lower anticancer activity than 1,3,6,8-tetrahydroxyxanthone ( $IC_{50} = 9.18 \mu\text{M}$ ) due to the presence of 4-hydroxyl which was inactive as aforementioned above.

We also compared the anticancer activity of hydroxyxanthone with doxorubicin as the positive standard representing the commonly used anticancer drug for the HepG2 cancer cell line. Among twenty-two hydroxyxanthone derivatives, only eleven hydroxyxanthones, i.e., 1-hydroxyxanthone, 1,6-dihydroxyxanthone, 1,7-dihydroxyxanthone, 2,5-dihydroxyxanthone, 3,5-dihydroxyxanthone, 1,3,5-trihydroxyxanthone, 1,3,6-trihydroxyxanthone, 1,3,7-trihydroxyxanthone, 1,3,6,7-tetrahydroxyxanthone, 1,3,6,8-tetrahydroxyxanthone, and 1,3,4,5,6-pentahydroxyxanthone, exhibited higher anticancer activity ( $IC_{50} = 9.18\text{--}45.9 \mu\text{M}$ ) than doxorubicin





**Figure 6.** (a) Three-dimensional and (b) two-dimensional structure of 1,3,6,8-tetrahydroxyxanthone on the active site of EGFR protein receptor

( $IC_{50} = 46.9 \mu M$ ). Their chemical structures are shown in Figure 3.

Among this group, it can be known that in general, the monohydroxyxanthone and trihydroxyxanthone gave weaker anticancer activity than dihydroxyxanthone. The dihydroxyxanthone gave weaker anticancer activity than tetrahydroxyxanthone and pentahydroxyxanthone. Therefore, the general order of the anticancer activity of hydroxyxanthones is monohydroxy- < trihydroxy- < dihydroxy- < pentahydroxy- < tetrahydroxy-. Trihydroxyxanthone is expected to give a higher anticancer activity than dihydroxyxanthone, as well as the pentahydroxyxanthone is expected to exhibit higher anticancer activity than tetrahydroxyxanthone. However, the arrangement of hydroxyl groups seems to be critical as they shall not form intramolecular hydrogen bonds, thus lowering their ability to interact with the protein receptors of the HepG2 cancer cell line. In all, the 1,3,6,8-tetrahydroxyxanthone was found as the best anticancer agent against the HepG2 cancer cell line with an  $IC_{50}$  value of  $9.18 \mu M$ , which was 5.11-fold more active than doxorubicin, which was remarkable.

### 3.2. Molecular docking of hydroxyxanthone

To elucidate the anticancer mechanism of

hydroxyxanthone against HepG2 cancer cell line, the molecular docking studies of the most potent hydroxyxanthone, i.e., 1,3,6,8-tetrahydroxyxanthone was conducted against TopII $\alpha$ , EGFR, and PDGFR protein receptors. The molecular docking studies were performed through four consecutive processes, i.e., preparation of protein receptor and native ligand, geometry optimization of hydroxyxanthone, re-docking of native ligand, and docking of hydroxyxanthone derivative. The preparation of protein receptors is the first step to discard water molecules and native ligands from the crystallographical structure of each protein receptor. This step is necessary to obtain a free active site in the protein receptor to be docked with the 1,3,6,8-tetrahydroxyxanthone. The three-dimensional structure of 1,3,6,8-tetrahydroxyxanthone was drawn and optimized using the DFT-B3LYP method with a basis set of 6,31G, as this parameter was commonly used for heterocyclic compounds [35].

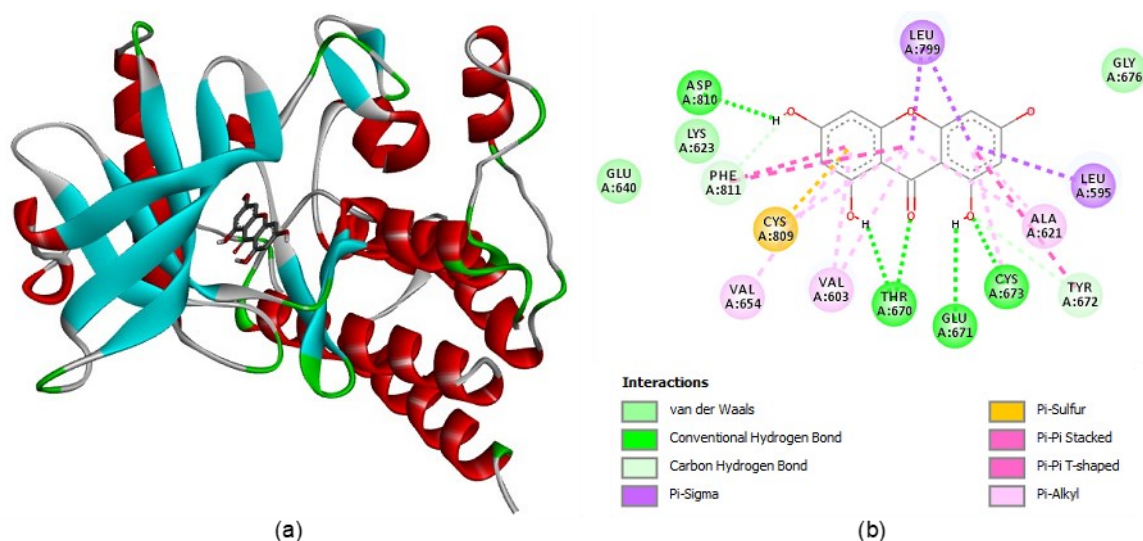
Afterward, the re-docking process was carried out in a  $50 \times 50 \times 50 \text{ \AA}$  grid box with 100 runnings of the Lamarckian Genetic Algorithm to elucidate the most stable conformation of native ligand in the active site of each protein receptor. After the docking process, the Cartesian coordinate of the native ligand was saved and compared to the original position as reported in the crystallographic

data. The superimposed three-dimensional structures of native ligand, i.e., mitoxantrone, erlotinib, and imatinib, on the active site of TopII $\alpha$ , EGFR, and PDGFR protein receptors are shown in Figure 4. The RMSD value for mitoxantrone, erlotinib, and imatinib was 1.22, 1.64, and 0.65 Å. These RMSD values were smaller than 2.00 Å demonstrating that the used docking parameters were valid.

The 1,3,6,8-tetrahydroxyxanthone was docked in the same position as the native ligand for each protein receptor. The three-dimensional and two-dimensional structures of 1,3,6,8-tetrahydroxyxanthone on the active site of the TopII $\alpha$  protein receptor are shown in Figure 5. From the three-dimensional structure, it was known that 1,3,6,8-tetrahydroxyxanthone was located near the DNA  $\alpha$ -helix and amino acid residues of chain A. Two-dimensional structure revealed that 1,3,6,8-tetrahydroxyxanthone interacted with Adenine12, Guanine13 and Cytosine14 nitrogen base residues, as well as Arginine503, Lysine505, and Alanine521 amino acid residues, through hydrogen bonds on the active site of TopII $\alpha$ . It was reported that the interactions with Adenine12 and Guanine13 were pivotal to stimulating the damage of cancer cells' DNA thus raising the apoptosis response [36][37]. Moreover, the 1,3,6,8-tetrahydroxyxanthone interacted with Glutamic acid522 through pi-anion interaction, with Arginine503 and Alanine521

through pi-alkyl interaction, as well as Glycine504, Isoleucine506, and Asparagine520 through van der Waals interactions. These interactions let the 1,3,6,8-tetrahydroxyxanthone gave the binding energy and binding constant of -25.48 kJ/mol and 34.3  $\mu$ M, respectively, with RMSD value of 1.85 Å on the active site of TopII $\alpha$  protein receptor (Table 2).

On the other hand, the three-dimensional and two-dimensional structures of 1,3,6,8-tetrahydroxyxanthone on the active site of EGFR protein receptor are shown in Figure 6. Two-dimensional visualization revealed that 1,3,6,8-tetrahydroxyxanthone interacted with Lysine721, Threonine766, Glutamine767, and Methionine769 on the active site of EGFR. The 1,3,6,8-tetrahydroxyxanthone also interacted with Leucine820 through pi-sigma interaction and Valine702 and Alanine719 through pi-alkyl interaction. Furthermore, van der Waals interactions with Leucine694, Methionine742, Leucine768, Proline770, Phenylalanine771, Glycine772, Threonine830, and Aspartic acid831 were also observed in the active site of EGFR protein receptor. It was reported that the interactions with key amino acid residues of EGFR, i.e., Glycine695, Glycine700, Glutamine767, Methionine769, Aspartic acid831, Glycine833, Arginine812, Asparagine818, and Tyrosine845 were pivotal to the suppression of cancer cell division [7][8]. The 1,3,6,8-tetrahydroxyxanthone generated the binding



**Figure 7.** (a) Three-dimensional and (b) two-dimensional structure of 1,3,6,8-tetrahydroxyxanthone on the active site of PDGFR protein receptor

energy and binding constant of -28.74 kJ/mol and 9.24  $\mu$ M, respectively, with RMSD value of 0.10 Å as listed in Table 2. This result indicated that 1,3,6,8-tetrahydroxyxanthone had the ability to inactivate the function of the EGFR protein receptor and suppress the division of HepG2 cancer cell line.

The three-dimensional and two-dimensional structures of 1,3,6,8-tetrahydroxyxanthone on the active site of the PDGFR protein receptor are shown in Figure 7. The results revealed that 1,3,6,8-tetrahydroxyxanthone interacted with Threonine670, Glutamic acid671, Cysteine673, and Aspartic acid810 on the active site of PDGFR. The 1,3,6,8-tetrahydroxyxanthone bonded to Tyrosine 672 and Phenylalanine811 through carbon-hydrogen bond, to Leusine595 and Leusine799 through pi-sigma interaction, to Cysteine809 through pi-sulfur interaction, to Valine603, Alanine621, and Valine654 through pi-alkyl interaction, and to Tyrosine672 and Phenylalanine811 amino acid residues through pi-pi stacked and pi-pi T-shaped interactions. It also interacted with Lysine623, Glutamic acid640, and Glycine676 through van der Waals interactions yielding the binding energy and binding constant of -30.42 kJ/mol and 4.71  $\mu$ M, respectively, with RMSD values of 1.85 Å (Table 2). It was reported that the interactions with Glutamic acid640, Cysteine673, and Aspartic acid810 residues were critical to deactivating the PDGFR function leading to the suppression of cancer cell proliferation [12]. From the molecular docking data, the 1,3,6,8-tetrahydroxyxanthone interacted with all these key amino acid residues at the hinge region  $\alpha$ C-helix DFG motif of the activation loop of PDGFR. It meant that 1,3,6,8-tetrahydroxyxanthone had the ability to deactivate the function of the PDGFR protein receptor and suppress the division of the HepG2 cancer cell line. Furthermore, it could be the reason that 1,3,6,8-tetrahydroxyxanthone exhibited the highest binding energy to PDGFR (-30.42 kJ/mol) over the other protein receptors (-25.48 to -28.74 kJ/mol) as it could bind to all key amino acid residues.

In summary, the 1,3,6,8-tetrahydroxyxanthone could bind to the active site of TopII $\alpha$ , EGFR and PDGFR protein receptors through *in silico* molecular docking studies. The results could be used to understand the mechanism of action of

1,3,6,8-tetrahydroxyxanthone as the anticancer drug against the HepG2 cancer cell line. The experimental *in vitro* MTT assay showed that 1,3,6,8-tetrahydroxyxanthone exhibited the IC<sub>50</sub> value of 9.18  $\mu$ M, which was much more active than doxorubicin (IC<sub>50</sub> = 46.9  $\mu$ M). This anticancer activity may be caused by the simultaneous effect of 1,3,6,8-tetrahydroxyxanthone to interact with the active site of TopII $\alpha$ , EGFR and PDGFR protein receptors. Interaction of 1,3,6,8-tetrahydroxyxanthone with Adenine12 and Guanine13 nitrogen bases on the active site of TopII $\alpha$  led to suppression of the DNA replication and transcription of cancer cells [36][37]. Meanwhile, the interactions of 1,3,6,8-tetrahydroxyxanthone with Glutamine767 and Methionine769 through hydrogen bonds, as well as Aspartic acid831 through van der Waals interaction, on the active site of EGFR caused the less signal for the cancer cells to proliferate, differentiate and survive [7][8]. On the other hand, the ability of 1,3,6,8-tetrahydroxyxanthone to interact with Cysteine673 and Aspartic acid810 through hydrogen bonds on the active site of PDGFR protein receptor, as well as with Glutamine640 through van der Waals, suppress the regulation of cancer cell to migrate, survive and proliferate [12].

All these mechanisms led to the death of cancer cells through the apoptosis mechanism; thus, it was reasonable if 1,3,6,8-tetrahydroxyxanthone was the most potent anticancer drug candidate to treat the human liver adenocarcinoma cell line. Even though the proposed mechanism of action for 1,3,6,8-tetrahydroxyxanthone was similar to the doxorubicin one. The 1,3,6,8-tetrahydroxyxanthone has different molecular size, conformation, physicochemical properties, and pharmacokinetic profiles [38][39]. These differences may overcome the doxorubicin resistance in some cancer cells, as reported by other research groups [40][41].

#### 4. CONCLUSIONS

In conclusion, the anticancer activity of hydroxyxanthones against the human liver carcinoma (HepG2) cell line depends on the number and position of the hydroxyl group. Xanthone with no hydroxyl substituent gave low anticancer activity (IC<sub>50</sub> = 85.3  $\mu$ M). However, the

presence of 1-hydroxyl substituent enhanced its anticancer activity ( $IC_{50} = 43.2 \mu M$ ). In contrast, the presence of either 4-hydroxyl or 7-hydroxyl demarcated the anticancer activity; thus, it was not recommended for the liver cancer drug design based on the structure of xanthone derivatives. Further investigation reveals that the additional hydroxyl groups at the left aromatic ring of 1,3-dihydroxyxanthone structure enhanced its anticancer activity. The 1,3,6,8-tetrahydroxyxanthone was found as the best anticancer drug among the evaluated hydroxyxanthenes with the  $IC_{50}$  value of  $9.18 \mu M$  and it exhibited 5.11 times stronger anticancer activity than doxorubicin as the commercially used anticancer drug, which was remarkable. Molecular docking studies revealed that the 1,3,6,8-tetrahydroxyxanthone could bind to the active site of TopII $\alpha$ , EGFR and PDGFR protein receptors with a binding energy of -25.48, -28.74, and -30.42 kJ/mol, respectively. The RMSD values (0.10–1.85 Å) were less than 2.00 Å demonstrating the validity of the molecular docking approach. Interaction of 1,3,6,8-tetrahydroxyxanthone with nitrogen bases on the active site of TopII $\alpha$ , as well as with amino acid residues on the active site of both c-KIT protein kinase receptors, led to simultaneous mechanisms to the death of cancer cells through apoptosis mechanism. These findings are important to guide the researchers to design and develop more potent anticancer drugs in the future.

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## Conflicts of Interest

The author(s) declare no conflict of interest.

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